

The Effects of Early Life Stress on Stress Induced Binge Eating Later in Life

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Abstract

Research has shown that stress not only affects our food intake but early life stress can affect this stress/eating relationship later in life as well. To study this, rats were subjected to early life stressors beginning on postnatal day 28 which consist of the adolescent phase of the rats lives, stressors consisted of elevated platform, damp bedding and restraint. On postnatal day 50, when rats had become adults they were then subjected to a mild electrical foot shock, after which, their intake of high palatable food was measured. Results demonstrate that rats who received early life stress ate less after the shock ($\bar{x} = 1.95g$) than rats who had not received early life stress ($\bar{x} = 3.50g$). These results suggest that early life stress increases rats' sensitivity to stressors, thus reducing their stress related binge eating tendencies. Results obtained in this study demonstrate a potential factor that causes rats to become stress under eaters or stress over eaters.

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The Effects of Early Life Stress on Stress Induced Binge Eating Later in Life

The interaction between early life stress and stress induced binge eating later in life is an important area of study as it can aid in understanding the development and etiology of eating disorders (Hancock et al., 2005) as it has been proven to be a large contributing factor to many other disorders (Martins et al., 2011). New to the DSM, binge eating disorder is classified as eating an excessive amount of food in a short period of time during the absence of hunger and with no attempt at weight control such as with bulimia nervosa (American Psychiatric Association, 2013). Since binge eating was only recently classified under eating disorders in the DSM-5 (American Psychiatric Association, 2013), it had not been as widely studied as other eating disorders such as anorexia nervosa or bulimia nervosa (Fairburn, 2002). The present study aims to contribute to the current literature and to determine whether or not early life stress does in fact influence vulnerability to stress induced binge eating. An animal model is utilized in the current study as it allows for more experimental control and fewer extraneous variables than with human participants. Furthermore, animal models have been proven to be very useful in helping to determine physiological reasons that aid in explaining human behaviours such as binge eating (Oswald et al., 2010), which may later help in determining treatment options (Hancock et al., 2005).

Stress can be defined as a bodily response to a generalized or specific danger that threatens to overwhelm or does in fact overwhelm the body's ability to maintain homeostasis (Cameron et al., 2011). When a person's or an animal's homeostasis is threatened by danger, such as a stressor, the body's "fight or flight" response kicks in. Regardless of the outcome one chooses, the body's sympathetic nervous system becomes activated, which leads to a reduction in digestion and typically, appetite suppression. For this reason, when most species are under stress,

they tend to reduce their food intake and be stress under eaters (eating less than they do when no stressor is present) (Torres et al., 2007). However studies have shown that some people actually over eat when stressed as opposed to under eat (which is the expected outcome) (Wolff et al., 2000). Though it is not completely certain why some people are stress under eaters whereas others are stress over eaters, there have been a few theories which have previously been studied to try and help explain this phenomenon. Some of the studies were looking at stress as a contributing factor to the differentiation of stress over eating and stress under eating; whereas other studies were exploring other possible explanations. Additionally animal models are often utilized to aid in explaining human behaviours such as these (Oswald et al., 2010). Low self-esteem for example, has been an area of study which seems to influence eating disorders; however binge eating disorder has not been a particular topic that has been explored during these studies, though it may be a factor (Ruggiero et al., 2008). Furthermore, higher levels of perceived stress as well as negative mood seem to be correlated to binge eating, unfortunately cause and effect in these particular studies cannot be established as the experiments are generally observational and not experimental (Wolff et al., 2000). Also, in an animal study using rats, past caloric restriction seemed to possibly be a factor that influenced stress induced binge eating (Boggiano et al., 2005) however not all other studies have been able to replicate this significant difference (Hancock et al., 2005). Another factor, namely, early life stress has been theorized as a variable which may influence stress induced binge eating, which will further be explored in this study.

A theory to help explain the influence of early life stress on behaviour is the widely known fact that many neurons and brain connections are formed during childhood and adolescents, marking these early ages as crucial in the brains development. These brain

connections influence how a person reacts in certain circumstances later in their life (Weiss, Wagner., 1998). Therefore early life events and stress help to form who a person is and how they behave. Furthermore, it is important to pay attention to puberty cycles of the species that is being studied as this will give us an idea of the sensitive periods when stressors would have the most impact on the development leading to long lasting changes. With respect to rats this “window” is a lot smaller in comparison to humans due to their short life cycles. Therefore there is only a small window of opportunity to subject rats to early life stress in their pre-adult development phase. Postnatal days (PND) 28 through 30 are 3 consecutive days immediately before the puberty cycle begins. PND 34 to 36 marks the middle of the female rat puberty cycle. By PND 40 through 42, the female rat’s puberty cycle has ended. Clearly when using rat models it is important to expose them to early life stress within the first weeks after their birth as their puberty cycle, marking adolescence, is very short.

Although there are many theories regarding what may possibly cause stress induced binge eating more research needs to be done before it can be understood which ones have to strongest support, as most of the current literature utilizes quasi experimental designs and therefore cannot infer cause and effect. The current study aims to help to make sense of some of these uncertainties regarding stress induced binge eating, such as whether or not early life stress does in fact influence ones vulnerability to stress induced binge eating. Therefore this study utilizes an experimental model in order to manipulate the variables so that possible cause and effect statements may be made. A system that plays a large role in stress the stress-response and may help to further explain theories of stress induced binge eating is the hypothalamic pituitary adrenal (HPA) axis.

Hypothalamic Pituitary Adrenal Axis

When various species' bodies (such as humans and rats) are under extreme stress, they activate a hormonal system called the hypothalamic pituitary adrenal axis (HPA axis). During the activation of this stress response, the hypothalamus receives a stress signal and releases a hormone known as corticotrophin-releasing hormone (CRH) (Smith et al., 2006). This in turn triggers the pituitary gland which itself secretes adrenocorticotrophic hormone (ACTH). The pituitary gland is a part of the adrenal gland which releases epinephrine once activated by stress. This hormone works to activate the sympathetic nervous system (Smith et al., 2006). Furthermore, the ACTH that is secreted by the pituitary gland then triggers the adrenal cortex which releases cortisol, leading to secretion of glucocorticoids. In the final phase of the HPA axis, cortisol works to reduce the amount of CRH being released by the hypothalamus, creating a chain of events which all work together to reduce the amount of hormones being secreted throughout the HPA axis (Smith et al., 2006).

The activation of the sympathetic nervous system is known to decrease appetite. Therefore under normal levels of stress most species are under eaters (Torres et al., 2007). However, chronic stress has been associated with the hyper activation of the HPA axis, leading to increased secretion of cortisol in the system resulting in more glucocorticoids. Increased glucocorticoids have been seen to damage the hippocampus which in turn reduces memory capacity, leading to the body's inability to formulate proper stress reactions thus using inappropriate coping mechanisms when stressed (Sapolsky, 2000). Therefore, it may be that the body's inability to formulate proper coping mechanisms to stress leads to the increased eating. Furthermore, reduced volume of the hippocampus has been associated with various disorders including mood disorders (Sapolsky, 2000). Therefore, the HPA axis may prove to be useful in

helping to understand stress overeating as it plays an important part in the body's stress reaction and has been associated with other various disorders.

Stress induced binge eating has been an area of study with a lot of literature looking at different factors which may contribute to this disorder, however nothing has been very explanatory. Early life stress is clearly a variable that affects many disorders in the DSM including substance addictions (Kosten & Kehoe, 2007) as well as mood and anxiety disorders (Heim et al., 1997) among many others. Therefore early life stress may prove to be a useful area of study to help explain vulnerability to stress induced binge eating. Thus far not very many studies have explored animal models of early life stress and stress induced binge eating since binge eating is still such a new disorder (American Psychiatric Association, 2013). Furthermore, since early life stress seems to be a factor that influences many other disorders in the DSM, it is a potential factor as a possible explanation for binge eating disorders. The main article within the previous literature that examines the effects of early life stress on stress induced binge eating is in regards to level of maternal care influencing vulnerability to stress induced binge eating. However many other studies have documented the effects of early life stress on multiple different behavioural responses other than stress induced binge eating, which are equally of interest to this topic as they may help us determine the extent to which early life stress has an impact on rats.

Effects of early life stress on behavioural responses.

In a study by Hancock, et al. in 2005 they attempted to determine if differences in maternal care affected the rats' vulnerability to stress induced binge eating. To conduct this study 24 female rats and their pups were observed during the first 7 days postpartum to determine the

level of maternal care all pups were receiving. Mothers lick and groom (LG) frequency was used to determine the level of maternal care that their pups received. All litters were classified within one of three groups; low LG, mid LG and high LG. Low LG rats were considered to be the high stress group and high LG rats were considered to be the low stress group. Once the pups had gotten older they were each subjected to a foot shock, after which their intake of high palatable food was measured.

Results indicate that rats in the low lick/groom group (high stress) ate significantly more than the high lick/groom group (low stress) after the foot shock. This study supported the idea that early life environment shapes vulnerability to stress induced binge eating. However this study was quasi experimental due to the fact that the rats were not randomly assigned to stress groups as level of maternal care was used as the independent variable. Accordingly, it is possible that the rats may have carried a genetic trait for either stress over eating or stress under eating. Consequently it does not allow the author to assume that the low level of maternal care was in fact the leading variable that caused the rats to over eat. Furthermore, genetics may have not been the only factor that caused the rats to over eat but it may have also been the cause of the low levels of licking and grooming from the mother. For this reason more research is needed to determine if early life stressors, other than maternal care, influence vulnerability to stress induced binge eating.

As well, there is reason to believe that early life stress may influence stress induced binge eating or even stress related reduction in eating after a stressor later in life since early life stress has been seen to influence other behavioural responses in rats other than binge eating. In a study done by McCormick et al. in 2007, they attempted to determine if social stress in adolescents affected anxiety-like behaviours in rats later in life. For this study they gathered both male and

female rats on postnatal day (PND) 22. All pups were placed in 1 of 3 groups; adolescent social stress, no-stress control, and acute stress control groups. Each rat was then frequently subjected to their corresponding level and type of stress. On postnatal day 45 rats anxiety behaviour was measured using an elevated plus maze task. In such a task the rat is placed in the centre of an elevated x shaped platform. Two of the maze arms were closed in with walls on both sides and the other two arms were open with no walls. Results showed that rats previously exposed to social stress exhibited fewer anxiety like behaviours than did either of the control groups, meaning they spent more time in the open arms than did the other groups of rats. This study helped determine that stress in adolescents does in fact influence behavioural responses to stress later in life as it concluded that early life stress does influence rats' anxiety like behaviours. Therefore stress in adolescents is a very plausible explanation for rats' vulnerability to stress related eating behaviours later in life.

Similarly, in a study by Rodriguez, et al. in 2013 they found similar changes in behavioural responses towards stress when they observed stress in adolescents and novelty seeking. For this study 33 male and female rats were acquired. They were subjected to stressors following their respective pre-puberty and puberty cycle, to insure that stressors were administered at all stages of adolescents. All rats received physical stressors at these times as opposed to social stressors which were used in the study by McCormick. Three physical stressors were utilized in this experiment; the first consisted of being placed in a novel location for 5 minutes. For the second stressor rats were placed in a closed box under a bright light for 25 minutes and were exposed to trimethylthiazoline (TMT) which is a strong odour. The final stressor consisted of being placed in an elevated platform for 25 minutes under a bright light. Rats' anxiety like behaviours were again measured using an elevated plus maze task. Results

showed that rats exposed to stress in adolescents demonstrated more risk taking behaviours than those who had not received the early life stressors. Again helping to demonstrate that early life stress, including physical stressors, do in fact influence behavioural responses to stressors later in life as Rodriguez was able to conclude that early life stress influences rats' anxiety like behaviours, leading to more risk taking in those who had received the early life stress, as opposed to the rats who had not.

Finally, in a study done by Chaby, et al. 2013 they observed changes in cognitive bias and coping response as a result of stress in adolescents. In this study, all the rats in the stress group were subjected to both physical and social stressors. Physical stressors consisted of placing the rats in a smaller cage for 4 hours, damping the rats bedding with 200ml of water for 6 hours and tilting the rats home cage on a 30° angle for 6 hours. Social stressors consisted of isolation, in which rats were separated from the rats that are normally housed with for an hour and a half, second social stress consisted of crowding for 4 hours in which extra rats were placed in the home cages. The final social stress consisted of placing rats in a cage full of used bedding from other older rats for 12 hours.

In Chaby's study, once the rats had gotten older they observed their cognitive bias, which is defined as misleading cognitions that impact the rats' decision making. Moreover, coping responses was also observed in all rats. Results showed that rats exposed to stress as adolescents demonstrated more of a cognitive bias than those in the control group. Furthermore they found that rats in the experimental group were more likely to partake in exploratory behaviours compared to their controls. This study demonstrates that not only anxiety like behaviours are influenced by early life stressors, rather many behavioural responses are affected by being influence to both physical and social stress during adolescents. This was demonstrated using the

studies conducted by McCormick, Rodriguez and Chaby in which behavioural responses were affected by first having been exposed to early life stressors. Therefore there is reason to believe that early life stress will in fact influence the occurrence of stress related eating disturbances later in life due to high exposure to cortisol, during a crucial phase of development, as it has already been seen to affect various other behavioural responses in rats.

Effects of Stress Sensitization

Though the study conducted by Hancock and others like it, seems to point in the direction of stress induced binge eating after being exposed to early life stress, there have been studies conducted in the past which may suggest that the rats become sensitized to the stressors possibly causing them to under eat (Servatius et al., 1995). In a study conducted by Servatius et al., in 1995, he examined rats delayed startle sensitization after being exposed to a stressor. For this study Servatius randomly assigned his rats to one of three groups. They were either assigned to the 3 days of tail shock group, the one day of tail shock group or the no stress control group. Rats' startle response was measured 4 days post stressor as well as 7 and 10 days post stressor. Results indicate that rats who received the more chronic stress (3 days of tail shock) had become sensitized to stressors and therefore displayed more exaggerated startle response than either of the other 2 groups. This study then led to the theory that rats previously exposed to chronic stress experience sensitization to stressors later in life, causing them to have a more exaggerated response to the stressor compared to those who had not previously been exposed to chronic stressors (Servatius et al., 1995). This then allows for the assumption that rats exposed to chronic stress in adolescents may have an exaggerated stress response to mild stressors. Where as normal rats may simply demonstrate slight changes in eating behaviours after a mild stressors, rats previously exposed to chronic stressors may treat these mild stressors as extreme stressors,

therefore allowing for their HPA axis to activate, leading towards a reduction in food intake all together (Servatius et al., 1995., Ahn & Phillips, 2012).

Present Study

In light of the literature outlined above, the present study aims to determine if physical stress during the adolescent phase of the rats' lives, specifically following a pre-puberty and puberty cycle, will change the rats' vulnerability to stress induced binge eating later in life. Because of the effects that early life environments have on the development of the brain and because of the impacts that large amounts of cortisol have on the brain, we hypothesize that the occurrence of early life stress will interact with the later life stress, leading towards a change in eating behaviours. The current study aims to further explore in which direction eating will go. There are currently theories for both an increase and a decrease in eating following a stressor after previously being exposed to chronic stress earlier on in life. The theory for the increase in food consumption after a stressor is that early life stress causes the rats to become hyper sensitized to stressors leading to the manifestation of stress related behaviours such as binge eating and greater vulnerability to stress later in life (Hancock et al., 2005). The theory as to why the rats may decrease their food consumption after a stressor is that while mild stressors (such as foot shocks) have been seen to increase food consumption in rats, in contrast rats previously exposed to mild stressors will become sensitized to them, thus reducing their intake of HP food (Ahn & Phillips, 2012).

In terms of the hypotheses for the main interactions, we hypothesize that early life stress will cause a change in HP food intake later in life as theory suggests that early life stress increases pathological eating later in life (Hancock et al., 2005). Furthermore we hypothesize

that the later life stress (foot shock) will cause a decrease in HP food intake as the theory suggests that stress causes the rats to under eat as the sympathetic nervous system activates and reduces hunger (Torres et al., 2007).

Methods

Subjects

The current study utilized 24 Long-Evans female rats that arrived at the animal colony on postnatal day (PND) 21. The rats were ordered from the Charles-Rivers colony. Upon arrival all rats were weighed individually. All rats weighed in between 40 and 70g. Once in the animal colony they were individually housed in plastic, transparent, bedded cages with free access to rat chow (Laboratory Rodent Diet 5001; LabDiet®; 4.0kcal/g, 59.8% carbohydrates, 4.5% fat, 23.4% protein) and water for 7 days prior to experiment. Each of the rat cages contained small plastic red tubes which were added as environment enhancers. All rats were acclimatized to a 12-h light/dark cycle throughout the length of the experiment. Using matched random assignment, based on weight of rats on post natal day 22; all rats were placed into either a treatment or a control group. Rats in the treatment group were subjected to stressors beginning on postnatal day 28, whereas rats in the control group remained in their housing, stress free on these days. All testing took place during the rats light cycle, between 8 a.m. and 8 p.m.

Materials

For one of the various early life stressors an elevated platform was used which consists of a transparent Plexiglas open field (40.5 x 40.5 x 31.5 cm) that was propped up 100cm off the ground using four wooden legs (2.5 x 2.5 x 100). Subsequently, restraint tubes consisting of transparent cylindrical Plexiglas containers with multiple ventilation holes and adjustable black

ends were also used for the early life restraint stress. For the later life stress a shock equipped operant chamber (30cm x 20cm x 25cm) with an electrified metal grid floor was used. The shock equipment was triggered manually by the experimenter and followed strict time intervals using a stopwatch.

In this experiment Reese Peanut butter chips (Hershey's® Reese peanut-butter chips; 61 5.2kcal/g, 47% carbohydrates, 28% fat, 21% protein) were used as the high palatable (HP) food during the final phase of testing. Various experiments in the past used these peanut butter chips or other peanut flavoured reinforcements in similar experiments (Hancock et al., 2005). They were used because the rats consumed a large amount of them in a short period of time as they were a more desirable food because it is higher in fats and carbohydrates than regular rat chow (Oswald et al., 2010). Peanut butter chips were given to the rats in their home cages on shock days as well as on control days. All rats received the peanut butter chips in a small ceramic bowl. Furthermore, all food was measured using the same Sunbeam digital scale during each test day.

Procedures

Early Life Stress. The early life stress was administered to those in the experimental group on postnatal days 28-30, 35, 36, 40, 42. The first stressor consisted of damp bedding, which was administered on postnatal days 29 and 40. Damp bedding was chosen as one of the early life stressors as it was used in the study by Chaby et al., 2013 and was found to be efficient in stressing the rats. For this stress rats were temporarily placed in a plastic container while 200ml of water was mixed into roughly 2/3 of their used bedding. Rats were placed back into their home cages with ad libitum access to food and rat chow. They remained in the damp bedding for 6 hours each. Once the 6 hours had elapsed, rats were again removed from their

cages temporarily and placed in a plastic container while their dirty bedding was changed with clean bedding. Rats were then placed back into their home cages. Control rats also had their bedding changed on these days.

The second stressor consisted of an elevated platform which rats were subjected to on postnatal days 28 and 35. Rats were subjected to the elevated platform as it was previously used in a study by Rodriguez et al., 2013 and was found to be a stressful environment for the rats to be subjected to. For this stress each rat was removed from their home cages one at a time and taken to a nearby behavioural testing room containing the elevated platform apparatus. Rats were placed into the elevated platform for 30 minutes each with no food or water. At the end of the 30 minutes each rat would then be brought back to its home cage. The elevated platform was cleaned with lemon quat, which is a commonly used cleaner in the animal colony, and then thoroughly dried between each rat to ensure that the scent from the previous rat did not remain in the elevated platform as it may distract the present rat in the apparatus.

The final stressor consisted restraint which was administered on postnatal days 30, 36 and 42. Restraint was used as one of the early life stressors as it was used in a study by Fachin et al., 2008, and was seen to be effective in stressing the rats. For this stressor rats remained in the room where they were housed, they were simply transported to a nearby table. Each rat was individually placed in a restraint tube which restricted their movement. Tubes were placed on a metal table with a blanket underneath the tubes to prevent them from rolling and to insure that the cold from the metal table did not affect the rats. Each rat was placed in a restraint tube for 45 minutes and then removed and placed back into its home cage. Tubes were cleaned with lemon quat; they were then thoroughly dried between rats.

During each of the early life stressors, all rats' behavioural stress responses were observed to ensure that the rats were in fact being stressed. Behaviours such as lack of grooming, freezing, differences in posture, vocalization and increased avoidance of handling as well as scratching and biting during handling were all monitored as they demonstrate stress signs in the rats. These behaviours are all stress responses as defined by the Canadian council of animal care (Canadian Council on Animal Care. 1993). If any rat were to demonstrate extreme stress behaviours, indicating that their wellbeing was in danger, they were immediately removed from the stress and placed back into their home cage.

Habituation to Food Restriction. To insure that the rats were habituated to the food restriction during the final phase of testing, all rats were habituated to being food deprived overnight. To do this, all rats had their food removed from their cages on postnatal days 32, 37 and 43 from 8p.m. till 8a.m. the next morning. During the food restriction period all rats continued to have ad libitum access to water, as they would have such access during the final phase of testing.

Habituation to High Palatable Food. On postnatal days 45-47 and 49, all of the rats were habituated to the high palatable food (HP food) to insure that they had a chance to overcome their neophobia. On postnatal days 45 and 46 all rats received roughly 8g of HP food in a small ceramic bowl overnight, from 8p.m. till 8a.m. the next morning. Rat chow was removed from all cages during habituation however rats all had ad libitum access to water at this time. HP food intake was measure at the end of both nights. On postnatal days 47 and 49, each rat was given a premeasured unlimited amount of HP food in small ceramic bowls for 2 hours starting at 8a.m. till 10a.m. to simulate the length of time which they would receive the HP food during the final phase of testing. During this time the rat chow was again removed from the

cages and the rats re continued to have ad libitum access to water. At the end of the 2 hour period, the food was measured to determine a baseline of HP food intake and to insure that all rats had overcome their neophobia.

Later Life Stress. Later life shock cycle began on post natal day 50. All rats were placed into one of two groups; each group had two shock days as well as two control days. Groups were counterbalanced with rats who had not received the early life stress and those that had. On stress days rats were each individually transported to a room with the shock equipped operant chamber containing a metal rod flooring. Each rat was then shocked four times with 63V of electricity (this is a mild shock, but still uncomfortable for the rat) for 3 seconds each of which were followed by a 15 second pause. At the end of the shock cycle each rat was brought back to its home cage and immediately given a premeasured amount of unlimited HP food. On control days the rats remained in their home cages, stress free and given a predetermined amount of unlimited HP food. HP food intake was measured for both groups after 15 minutes of receiving the HP food as well as after 30 minutes, 1 hour and 2 hours. There were four testing days in total; each group had two shock days as well as two control days. Each test day was followed by one day of rest. Before each test day the rats were food restricted starting from 8p.m. the night before till 8a.m. on the test days when they were given the high palatable food. At the end of the each 2 hour HP food consumption period, all rats were given back their rat chow. See Figure 1 for visual timeline of procedures.

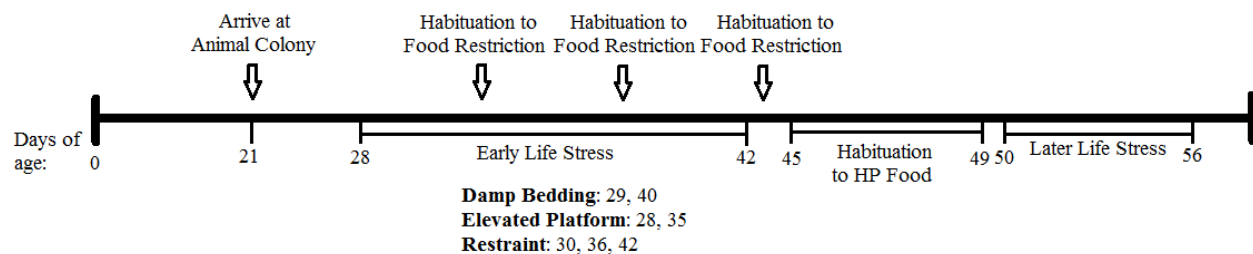
Rat Chow Intake. The later life stress procedure was again repeated for two additional days. However when rats were returned to their home cages after being shocked, or remained there on control days, they were given their regular rat chow to eat as opposed to HP food. The two additional days of later life stress with the rat chow food intake were to insure that there

would be no supplementary significance found with the rat chow as opposed to the HP food. Results of the two additional days of testing indicate that there was no significant interaction of early life stress on chow intake after a later life stress. These results further elaborate the findings of previous studies that rats are more motivated to consume the HP food as opposed to regular rat chow.

Analysis

All Statistical analyses were performed using SPSS (Statistics Package for Social Sciences) version 20. To examine the interaction effects of early life stress on stress induced binge eating later in life a mixed design two way analysis of variance (ANOVA) was performed to determine if there was a significant interaction effect of early life stress and later life stress on HP food intake across all four testing days. Furthermore a one way ANOVA was used to determine whether there was a significant effect of early life stress on HP food intake. Lastly a one way ANOVA was again used to determine if there was a significant interaction of shock on HP food intake.

Figure 1. Timeline of Procedures



Results

Effects of Early Life Stress and Later Life Stress on HP Intake

A 2x2 mixed design ANOVA was utilized in order to determine whether there was any significant interaction between early life stress and later life stress on high palatable food intake. In this analysis rats who had received early life stress were compared to the rats who had not received the early life stress when shocked later in life and when not shocked later in life. Four separate ANOVAs were run for each time interval at which the intake of high palatable food was measured. The results indicate that there was no significant interaction effect between early life stress and later life stress on HP food intake after 15 minutes, $F(1, 46)=1.358$, $p>0.05$. Furthermore no significance was found at any of the other 3 time intervals at which the intake of HP food was measured. Table 1 depicts the mean intake of HP food at all 4 time intervals.

Table 1

Mean intake of HP food for all rats on shock days and on no shock days after 15 minutes, 30 minutes, 1 hour and 2 hours of receiving HP food.

		<u>15 minutes</u>	<u>30 minutes</u>	<u>1 hour</u>	<u>2 hours</u>
Shock	Experimental	1.9583	3.6250	5.2917	6.7083
	Control	3.5000	5.0417	6.5833	8.2917
No Shock	Experimental	2.7917	3.8750	5.5417	6.9583
	Control	3.2083	4.2083	6.0000	7.4583

($p>0.05$)

Though there was no significant interaction between early life stress and later life stress on HP food intake, the means seem to propose rats who received early life stress (experimental group) are eating less when shocked than the controls. Whereas the means of both groups when

not shocked seem to be similar. Thus suggesting that rats who receive early life stress might be eating less when shocked than rats that have not received the early life stress. Figure 2 depicts this possible relationship.

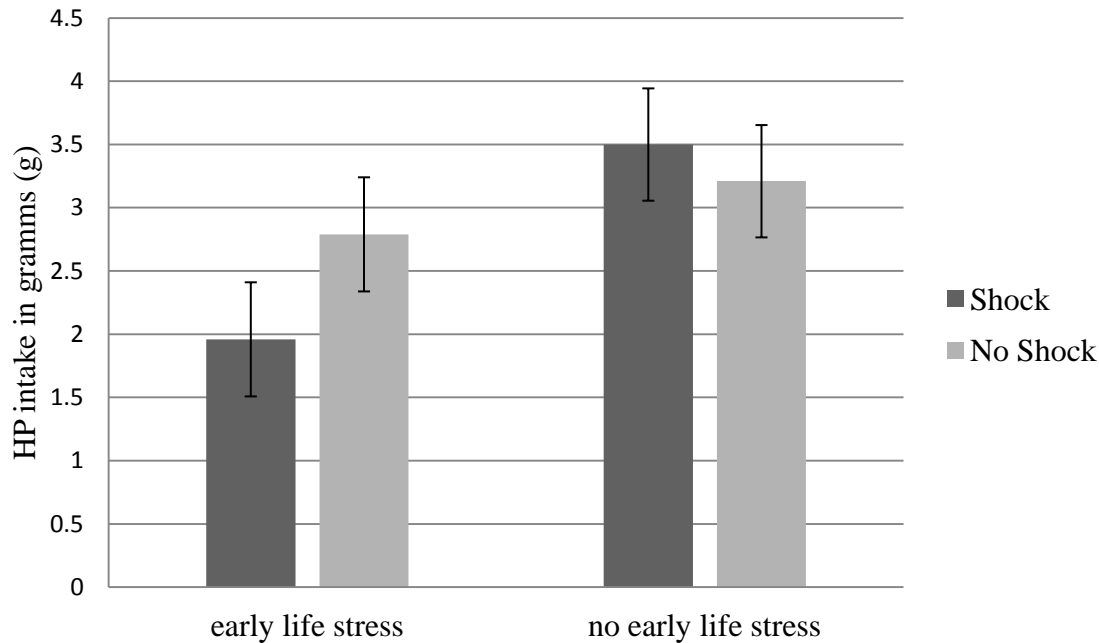


Figure 2. Mean HP food intake of all rats when shocked and not shocked after 15 minutes of receiving HP food. Error bars indicate standard error of the mean.

Effects of Early Life Stress on HP Intake

Four one way ANOVAs were used to determine if there was a significant effect of early life stress on HP food intake at all 4 time intervals. To conduct this analysis rats who received early life stress were compared to the control rats who had not received the early life stress based on their amount of HP intake, regardless of later life stress. There was a significant main effect of early life stress on HP food intake after 15 minutes $F(1, 46) = 5.699, p < 0.05, r = 0.33$. Furthermore there was also a significant main effect of early life stress on HP food intake after

30 minutes $F(1, 46) = 4.264$, $p < 0.05$, $r = 0.29$, and 2 hours $F(1, 46) = 4.813$, $p < 0.05$, $r = 0.31$.

Results for the main effect of early life stress on HP food intake are demonstrated in table 2.

Table 2

Mean intake of HP food of rats who received early life stress and rats who had no early life stress, after 15 minutes of receiving HP food, 30 minutes, 1 hour and 2 hours.

	<u>15 minutes</u>	<u>30 minutes</u>	<u>1 hour</u>	<u>2 hours</u>
Experimental	2.3750*	3.7500*	5.4167	6.8333*
Control	3.3542	4.6250	6.2917	7.8750

*Denotes significant difference compared to control group ($p < 0.05$)

The only time interval at which no significant difference in HP food intake was found between rats in the experimental group (early life stress rats) and those in the control group was at the 1 hour HP food intake measure. However the significance level at the 1 hour measure was at 0.069 which is close to the alpha level of 0.05, therefore though it was not significant it is worth mentioning, as it was close to significant. Ergo, rats who received early life stress eat significantly less than rats that had not.

Effects of Later Life Stress on HP Intake

Four one-way ANOVAs were used to determine if later life stress had a significant effect on HP food intake at all 4 time intervals at which the HP food was measured. To conduct this analysis, the amount of HP food intake rats consumed after receiving a shock and after not receiving a shock was compared, regardless of early life stress. There was no significant main effect of later life stress on HP food intake after 15 minutes $F(1, 46) = 0.315$, $p > 0.05$.

Furthermore no significant main effect of later life stress on HP intake was found at any of the

other 3 time intervals. Table 3 depicts the mean intake of HP food of rats when shocked and when not shocked at all 4 time intervals at which the HP food was measures.

Table 3

Mean intake of HP food of all rats after shock and no shock, following 15 minutes of receiving HP food, 30 minutes, 1 hour and 2 hours.

	<u>15 minutes</u>	<u>30 minutes</u>	<u>1 hour</u>	<u>2 hours</u>
Shock	2.7292	4.3333	5.9375	7.5000
No Shock	2.8788	4.0417	5.7708	7.2083

(p>0.05)

Discussion

The present study aimed to determine whether early life stress influenced rats' eating behaviours after a later life stressor. Results of the mixed design ANOVA indicate that there is no significant interaction between early life stress and later life stress on HP food intake. Therefore, the hypothesis which stated that physical stress during the adolescent phase of the rats' lives would change the rats' vulnerability to stress induced binge eating later in life, was not supported. However the means suggest an emerging trend that rats who receive early life stress are eating less when shocked compared to the rats who had not received the early life stress. Though the ANOVA did not determine significance, the emerging trend supports the hypothesis that early life stress changes eating behaviours after a later life stressor. This finding supports previous literature which suggests that prior exposure to stressors leads to long lasting sensitization to stress. For eating behaviours in particular, past literature suggests that mild stressors such as a foot shock, increases food consumption in rats. However, rats previously exposed to mild stressors will become sensitized to them, thus reducing their intake HP food (Ahn & Phillips, 2012). The reduction of HP food intake can be attributed to the activation of the sympathetic nervous system since the rats have been sensitized to mild stressors and now treat them as extreme stressors.

Furthermore when comparing the HP food intake of rats who received early life stress and those that had not regardless of later life stress, the rats in the experimental group were eating significantly less than the controls. Therefore the hypothesis which stated that early life stress will cause a change in HP food intake later in life was supported. However, since later life stress was not considered in this calculation it is important to note that it could have possibly been the shock that was driving the difference between the two groups. Since the means of HP

food intake seem to be consistently lower in rats who received early life stress specifically after a shock, it's important not to make the assumption that all rats who received early life stress are eating less regardless of later life stress.

Lastly in the results for the effects of shock on HP food intake, rats were seen to eat consistently the same amounts whether they had been shocked or not shocked at all 4 time intervals. Therefore the hypothesis which stated that later life stress (foot shock) would cause a decrease in HP food intake was not supported. Though previous literature does assume some change in rats' food intake after a stressor, it seems as though other factors in the current study prevented that change from occurring. For instance, most studies when observing stress under eating behaviours in rats, prefer to utilize regular rat chow as opposed to high palatable foods after the stressor as the rats do not have as much of an affinity to consume it (Calvez et al., 2011). Thus making it easier to observe the under eating. Therefore it might have been the use of HP food that resulted in the lack of difference between rats when shocked and when not shocked.

It is important to acknowledge that there were limitations to the study which may have contributed to the rejection of two of the three hypotheses. First of all, the sample size of the current study was particularly small. Other previous studies such as the one conducted by McCormick et al., utilized 100 female and 100 males rats in their study on the effects of adolescent social stress on anxiety. In this study McCormick et al., was successful in supporting his hypothesis that chronic stress in adolescents reduces anxiety like behaviours in rats. Therefore, had a larger sample size been used in the current study, there may have been a larger distinction in HP food intake between rats who received early life stress and those that had not. The rationale for using such a small sample size in the current study was based on the limited amount of time and resources that were available through the course of the research.

Additionally 24 female rats was the smallest sample size possible in which significance may have been found, however based on the results, it was not enough to demonstrate this particular phenomenon. Future studies on this topic should utilize larger sample sizes which mirror those of previous studies which found significance.

Another limitation which may have affected the results of the study is in regards to the transportation of the rats to the Laurentian colony. As opposed to breeding the rats for the experiment at the Laurentian colony, all the rats were flown to the destination from the Charles River's colony. The transportation of the rats lead to the inevitable stress all rats experienced for the duration of the move. Furthermore the environmental change from having all rats moved from one settlement to another is another source of stress all rats had to go through. Lastly another stressor all rats experienced was the individual housing. Prior to being brought to the Laurentian colony all rats had been group housed. However to minimize other extraneous variables, rats were individually housed upon their arrival at the Laurentian colony. Since rats have been seen to be social animals (Kiyokawa et al., 2011), isolation is a form of social stress. Considering all the stress rats experienced from their move from one colony to the other, it is obvious that all rats were subjected to some form of stress. Therefore, in the current study, the rats who were used as the no early life stress controls, did not experience the ideal stress free early life that would have made them the perfect controls. Consequently this is an extraneous variable that inevitably had an impact on the results. Future research on this topic should utilize rats' bread within the colony in which they will receive treatment. This will significantly reduce the amount of early life stress subjected to the rats who are supposed to experience a stress free early life.

Finally, one last limitation to the current study is the times at which all animals received their later life stress. Since only one shock equipped operant chamber is available at the Laurentian animal colony, rats did not all receive the later life stress at the same time. All rats were food deprived the night before the experiment beginning at 8 p.m. Socking began at 8 a.m. the next morning. All rats received their HP food somewhere between 8 and 9 a.m. However the rats that had to wait longer to receive the HP food, which was the first food they received all day, could have possibly been hungrier than those who received it promptly at 8 a.m., thus increasing their motivation to consume the HP food, and increasing their intake of the HP food. As a result, hunger levels of rats may not have been equal, the disparity in HP food consumption may not have been due to treatment, rather various levels of appetite. Future research on this topic should consider finding methods to insure that all rats are equally hungry when receiving the HP food, or insure that they are all shocked and fed at the same time.

The results obtained in the current study have aided in expanding on previous research. Firstly, when comparing these results and those of Hancock et al., 2005, there are clearly some conflicting findings. The current study seems to suggest that rats who received early life stress eat less after a later life stressor, whereas Hancock's results indicate that rats who received early life stress eat more after a later life stressor. However it is important to note that Hancock utilized a quasi-experimental design as maternal care was used as her independent variable and rats were not randomly assigned to their level of stressors. Additionally, when compared to stressors used in the current study such as restraint, level of maternal care seems to be a very mild stressor. Therefore Hancock's results may have been due to genetics or other extraneous variables as opposed to the level of stress they received. Furthermore Hancock's results are not generalizable to all early life stressors, only level of maternal care, since the rats in the current

study who received other early life stressors did not exhibit the same eating behaviours after a later life stressor.

Furthermore, results in the current study help to elaborate on the research conducted by Chaby et al., 2013, McCormick et al., 2008, and Toledo-Rodriguez et al., 2011. These studies all looked at the effects of chronic stress in adolescents on behavioural responses to stress later in life. However none of the behaviours looked at in these studies were in regards to eating. Therefore the results obtained in the current study demonstrate that chronic stress in adolescents does in fact have a long lasting effect on the rats eating behaviours later in life. Since these other studies were not looking at the effects of chronic stress in adolescent on eating behaviours, only on other behavioural responses to stress, this current study adds to the list of behaviours that are affected by chronic stress in adolescents.

Moreover when comparing the results obtained in the current study to those obtained by Servatius et al., 1995, these results help to further elaborate that chronic stress in adolescents sensitizes rats to stressors. In the study conducted by Servatius, he was observing rats' startle responses after they received chronic stress. His results indicated that the rats had in fact become sensitized to the stressors and thus exhibited more exaggerated startle responses. Similarly, in the current study, rats who experienced early life stress became sensitized to stressors and therefore when they were subjected to a stressor later in life they had a more exaggerated stress response. Therefore instead of over eating to the mild stressor, as was seen in previous studies in the past (Ahn & Phillips, 2012), they were sensitized and thus treated the mild stressor as an extreme stressor, in turn activating the sympathetic nervous system and reducing hunger which led to a reduction HP food intake. For that reason the current study further elaborates on the findings of

Servatius, that previous chronic stress sensitizes the rats to the stressors, leading to not only a more exaggerated startle response, but also a reduction in HP food intake.

Though the results obtained in this study did not demonstrate that early life stress causes a change in eating behaviours after a later life stress, there was a trend which suggested that rats who receive early life stress eat less after a later life stressor. Had the current study utilized a larger sample and possibly removed the extraneous variables, this trend may have demonstrated a significant difference between the experimental and the control groups. This then would demonstrate the impacts of early life stress and how it shapes our reactions to stress later in life, thus aiding in the explanation and differentiation of stress under eaters and stress over eaters. Furthermore it would imply that early life stress may play a role in the etiology of eating disorders since early life stress causes a greater reduction of HP intake due to a stressor later in life. Results would then demonstrate that early life stress affects eating behaviours later in life.

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